Original Article

Analysis of Allergens in 5 473 Patients with Allergic Diseases in Harbin, China

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Abstract

Objective To analyze the allergic status to common inhalant allergens and food allergens in clinical patients in Harbin in northeastern China and provide evidence to develop the prevention strategy of allergic disease.

Methods The data were collected from 5 473 patients with clinical suspected allergic diseases seeking medical care in the Second Affiliated Hospital of Harbin Medical University. Among these patients, 2 530 (46.2%) were males aged 0-86 years, the youngest was only 1 month old and 2 579 (47.1%) were young children and teenagers. The serum specific Immunoglobulin E (sIgE) to 14 kinds of common allergens and serum total IgE were detected by using AllergyScreen test (Mediwiss Analytic GmbH, Moers, Germany).

Results In 5 473 subjects the positive rate of sIgE was 33.1% (*n*=1 813). Cow milk (6.9%) and wheat (3.1%) were the most common food allergens, followed by house dust mite mix (12.5%) and mould mix (9.4%) and the age and gender specific differences in the positive rate were significant. For the children aged <7 years the positive rates to cow milk, beef-mutton, and egg white/egg yolk were high, but the positive rates to house dust mite mix, ragweed estragon, and mould mix were low (P<0.05). For the adults the positive rates to aeroallergens were high while the rates to food allergens were low.

Conclusion The results from this study showed that the food allergens in Harbin had geographic characteristics, which support the viewpoint that the environment factors play an important role in the incidence of allergic diseases. Also, the detection of sIgE and total IgE are essential to identify relevant allergens for the purpose of early diagnosis, management and prevention of allergic disease.

Key words: Aeroallergens; Food allergens; Specific IgE; Harbin; China

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INTRODUCTION

It is known that allergies is a common allergic disease which affects the quality of life of human being. In recent 20 years, the incidence of allergic diseases increased rapidly worldwide[1-2], resulting in substantial medical and economic burdens in both developed and developing countries[3]. Though it is not clear so far why did the incidence of allergic disease increase rapidly, it has been reported that the incidence of this disease is related with geographic locations and sub-populations[1], suggesting that genetic and environmental factors might play critical roles in the

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pathogenesis of allergic disease[4] and further epidemiological studies should be conducted in different sub-populations and/or in different geographic areas.

Because the most effective prevention of allergic disease is to avoid contacting or intaking allergens, it is essential to identify the suspected allergen for an atopic individual and the serum-specific immunoglobulin E (sIgE) assay is commonly used for this purpose and the test result can be used as index for diagnosis and therapeutic selection[5-6].

The incidence of allergic diseases is relatively lower in China compared with that in western countries, but less epidemiological studies on allergic disease have been conducted in China, especially in the northeastern China. So the aeroallergens and food allergens in Harbin were studied in this study.

MATERIALS AND METHODS

Subjects

Harbin, the capital of Heilongjiang province, is located in northeastern China at longitude 125°42′-130°10′ E, and latitude 44°04′-46°40′ N. and has an annual average temperature of 38.48 °F (3.60 °C). Administratively Harbin is composed of 8 districts (urban areas) and 10 counties (rural area) covering an area of 53 100 km^2. The population is about 10.64 million according to the nationwide census in 2010. Harbin is also an important production base of commodity grain (soybean, potatoes, flax, beet, rice, wheat, and vegetables) in China.

From August 2008 to July 2011, a total of 5 515 patients with clinical suspected allergic diseases seeking medical care in the Second Affiliated Hospital of Harbin Medical University were recruited in this allergen study by using sIgE assay. Finally 5 473 patients with complete data were selected for the further analysis and 42 patients with incomplete data were excluded. Of 5 437 patients, 4 806 (87.8%) were from the different out-patient departments of the hospital, including departments of dermatosis/STDs, pediatrics, respiratory disease, internal medicine, physical examination center, surgery, gerontology, ENT (ear-nose-throat) and others (Table 1).

The age of the subjects ranged from 0 to 86 years, the youngest was 1 month old and the oldest was 86 years old. The subjects were divided into eight

Table 1. The Patients' Sources of Department

Departments	Number of Patients	Percentage (%)
Dermatosis/STDs	2 996	54.7
Pediatrics	1 642	30.0
Respiratory disease	195	3.6
Internal medicine	138	2.5
Physical examination center	112	2.0
Surgery	91	1.7
Gerontology	60	1.1
ENT (ear-nose-throat)	33	0.6
Other departments	206	3.8
Total	5 473	100.0

age groups: 0-3 years, 4-6 years, 7-12 years, 13-19 years, 20-29 years, 30-39 years, 40-49 years, and over 50 years. Among the subjects 2 579 (47.1%) were young children and teenagers, and 2 530 (46.2%) were males. The collection and detection of samples collected from the patients lasted for nearly three years (August 2008-July 2011) and data analysis was completed in 2012. The study was approved by the Institutional Review Board (IRB) of the Second Affiliated Hospital of Harbin Medical University.

Methods

Six common aeroallergens and five food allergens were detected for all 5 473 patients. Aeroallergens included house dust mite mix (mixture of Derm. farinae and Derm. Pteronyssinus), short ragweed estragon, cat epithelium/dog epithelium, cockroach, mould mix (mixture of *Penicillium notatum*, branch spore mildew, *Aspergillus* fumigates and *Alternaria*) and grass. Food allergens included cow milk, beef-mutton, cashew-peanut-soybean, mango, and wheat. In addition, another three allergens including one aeroallergen [trees (mixture of cypress, elm, phoenix tree, willow, and cottonwood)] and two food allergens (egg white/egg yolk and fish-prawn-crab) were detected for 3 721 cases.

Serum allergen-specific Immunoglobulin E (sIgE) and total IgE of the patients were assayed by the AllergyScreen test (Mediwiss Analytic GmbH, Moers, Germany) according to the manufacturer's manual[7]. According to the results from AllergyScreen system sIgE were divided into six levels: grade 0 (<0.35

IU/mL), grade 1 (0.35-0.70 IU/mL), grade 2 (0.70-3.50 IU/mL), grade 3 (3.5-17.5 IU/mL), grade 4 (17.5-50 IU/ml), grade 5 (50-100 IU/mL), grade 6 (>100 IU/mL). The sIgE level>0.35 IU/mL was considered to be positive and significant reference range of serum total IgE was defined as more than 100 IU/mL.

Data Analysis

Data were classified and analysed by using the IBM SPSS Statistics version 19. For categorical values, chi-square test or Fisher's exact probability method was used to test positive rates to different allergens stratified by age and sex. Positive rates of sIgE of different allergens in 5 473 patients and in 3 721 patients were compared with standardized rates. *P*<0.05 was considered to be significant.

RESULTS

The overall positive rate of sIgE was 33.1% (1 813/5 437). The house dust mite mix and mould mix were most common allergens with the positive rates of 12.5% and 9.4%, respectively. In addition, 431 cases (7.9%) were sensitive to short ragweed estragon, 129 (3.5%) to trees, 152 (2.8%) to cockroach, 129 (2.4%) to cat epithelium/dog epithelium, 106 to grass (1.9%). Also, two most common food allergens were cow milk (6.9%) and wheat (3.1%), followed by beef-mutton (2.9%), egg white/egg yolk (1.6%), fish-prawn-crab (1.5%), cashew-peanut-soybean (1.5%) and mango (0.9%) (Table 2). Because trees, egg white/egg yolk and fish-prawn-crab were examined in only 3 721 of 5 473 patients, the positive rates of sIgE to the three allergens were standardized in order to avoid bias. The results showed that the standardized rates were similar to the test results.

An obvious association between age and the sIgE positive rates to house dust mite mix, ragweed estragon and mould mix was observed. The positive rates to these three allergens were low in those aged <7 years, but high in those aged 7 to 29 years. The positive rate to house dust mite mix decreased in those aged≥30 years. However, the positive rates to ragweed estragon and mould mix remained to be high in those aged <50 years. The sIgE positive rates to house dust mite mix and mould mix increased but the rate to ragweed estragon declined in those aged >50 years.

The positive rates to food allergens were slightly higher in young children groups. The positive rates to cow milk, beef-mutton, and egg white/egg yolk were relatively high in children aged<7 years (*P*<0.05), but declined in those aged>7 years. The rates to cow milk and beef-mutton remained to be low in those aged>20 years. There were no obvious changes in all age groups in three aeroallergens (cat epithelium and dog epithelium, cockroach, and grass) and three food allergens (cashew-peanut-soybean, mango, and fish-prawn-crab) (Table 2).

The overall positive rate was higher in males than that in females (67.3% *vs*. 47.4%, *P*<0.05). There were no obvious gender specific differences in the positive rates to allergens of mould mix, cat epithelium/dog epithelium, cockroach, and fish-prawn-crab, but the positive rates to other 10 allergens were higher in males than in females (Table 3).

The gender specific positive rates to allergens between two adjacent age groups were compared in the present study. The positive rates of sIgE to house dust mite mix, ragweed estragon and mould mix were higher In males than in females aged 0-3 years and 4-6 years (*P*<0.05). However, the positive rates to these three allergens were higher in females than in males aged 40-49 years and aged>50 years. There were also significant gender specific differences in the sIgE positive rates to 6 allergens (house dust mite mix, ragweed estragon, mould mix, cow milk, beef-mutton, and egg white/egg yolk) in those aged 4-6 and 7-12 years (Table 2).

There were no season specific differences in the positive rates to all allergens. 63.3% of patients have elevated total IgE and the number of patients detecting sIgE increased from 1 514 in 2008 to 2 281 in 2011.

DISCUSSION

During the past 25 years, the prevalence of allergic disease increased greatly worldwide, particularly in Western countries[8]. In China, it was reported that the prevalence of allergic disease is relatively lower compared with that in the US or Western Europe[9]. Globally, the difference in prevalence of allergic disease is shrinking now[10-11].

In our study the positive rates to aeroallergens ranged from 12.5% to 1.9% and the positive rates to food allergens ranged from 6.9% to 0.9%, which were relatively lower than those in South-east Asian[12-13]. The findings from this study revealed that the most common aeroallergen was house dust mite

Table 2. Positive Rates of Aeroallergens and Food Allergens in Patients Stratified by Age and Sex

Allergens	Number of Positive Patients (%)									*P*-value
	0-4 years	4-7 years	7-13 years	13-20 years	20-30 years	30-40 years	40-50 years	≥50- years	Total	
Number of Patients	**606**	**722**	**794**	**457**	**518**	**723**	**882**	**771**	**5 473**	
House dust mite mix	37 (6.1)$^{*\triangle}$	76 (10.5)$^{*\#\triangle}$	123 (15.5)$^{*\#\triangle}$	76 (16.6)	90 (17.4)$^{*\#\triangle}$	70 (9.7)$^{*\#\triangle}$	92 (10.4)$^{\#\triangle}$	118 (15.3)$^{\#\triangle}$	682 (12.5)	0.000**
Short ragweed estragon	19 (3.1)$^{*\triangle}$	45 (6.2)$^{*\triangle}$	79 (9.9)$^{*\triangle}$	51 (11.2)	64 (12.4)	72 (10.0)	64 (7.3)$^{\#\triangle}$	37 (4.8)$^{\#\triangle}$	431 (7.9)	0.000**
Cat epithelium and Dog epithelium	23 (3.8)	23 (3.2)	24 (3.0)	20 (4.4)	12 (2.3)	12 (1.7)	4 (0.5)	11 (1.4)	129 (2.4)	0.000**
Cockroach	9 (1.5)	11 (1.5)	16 (2.0)	17 (3.7)	24 (4.6)	21 (2.9)	31 (3.5)	23 (3.0)	152 (2.8)	0.023**
Mould mixing	35 (5.8)$^{*\triangle}$	71 (9.8)$^{*\#\triangle}$	104 (13.1)$^{\#\triangle}$	47 (10.3)	39 (7.5)	54 (7.5)	69 (7.8)$^{\#\triangle}$	96 (12.5)$^{\#\triangle}$	515 (9.4)	0.001**
Grass	4 (0.7)	13 (1.8)	24 (3.0)	13 (2.8)	12 (2.3)	16 (2.2)	14 (1.6)	10 (1.3)	106 (1.9)	0.010**
Cow milk	124 (20.5)	124 (17.2)$^{*\#\wedge}$	64 (8.1)$^{*\#\wedge}$	21 (4.6)$^{\wedge}$	12 (2.3)	12 (1.7)	8 (0.9)	14 (1.8)	379 (6.9)	0.000**
Beef- mutton	46 (7.6)	48 (6.6)$^{\#\triangle}$	33 (4.2)$^{\#\triangle}$	8 (1.8)$^{\triangle}$	6 (1.2)	10 (1.4)	2 (0.2)	5 (0.6)	158 (2.9)	0.000**
Cashew-peanut- soybean	10 (1.7)	12 (1.7)	12 (1.5)	5 (1.1)	7 (1.4)	8 (1.1)	18 (2.0)	9 (1.2)	81 (1.5)	0.643
Mango	1 (0.2)	5 (0.7)	12 (1.5)	1 (0.2)	4 (0.8)	7 (1.0)	15 (1.7)	5 (0.6)	50 (0.9)	0.126
Wheat	31 (5.1)	35 (4.8)	32 (4.0)$^{*\wedge}$	9 (2.0)$^{*\wedge}$	10 (1.9)	16 (2.2)	21 (2.4)	14 (1.8)	168 (3.1)	0.200
Number of Patients	**416**	**476**	**497**	**304**	**357**	**505**	**612**	**554**	**3 721**	
Trees	8 (1.9)	13 (2.7)	18 (3.6)	16 (5.3)	21 (5.9)	16 (3.2)	29 (4.7)$^{*\triangle}$	8 (1.4)$^{*\triangle}$	129 (3.5）	0.003**
Egg white and egg yolk	20 (4.8)	21 (4.4)$^{\#\triangle}$	7 (1.4)$^{\#\triangle}$	4 (1.3)	0 (0.0）	3 (0.6)	2 (0.3)	4 (0.7)	61 (1.6)	0.000**
Fish-prawn-crab	2 (0.5)	4 (0.8)	5 (1.0)	3 (1.0)	8 (2.2)	7 (1.4)	13 (2.1)	14 (2.5)	56 (1.5)	0.059

Note. Two-sided *P*-values: Chi-square test or Fisher's exact probability method for gender specific positive rates to allergens between adjacent age groups. *Males, $P<0.05$; $^{\#}$Females, $P<0.05$; $^{\triangle}$Total, $P<0.05$. Two-sided *P*-values: Chi-square test for positive rates of allergens among eight age groups. **Eight age groups, $P<0.05$.

Table 3. Comparison of Positive Rates of Aeroallergens and Food Allergens in Patients in Both Sexes

Allergens	Number of Positive Patients (%)			
	Male	Female	Total	*P*-value*
Number of Patients	**2 530**	**2 943**	**5 473**	
House dust mite mix	358 (14.2)	324 (11.0)	682 (12.5)	0.000*
Short ragweed estragon	238 (9.4)	193 (6.6)	431 (7.9)	0.000*
Cat epithelium and/Dog epithelium	66 (2.6)	63 (2.1)	129 (2.4)	0.255
Cockroach	75 (3.0)	77 (2.6)	152 (2.8)	0.435
Mould mix	220 (8.7)	295 (10.0)	515 (9.4)	0.093
Grass	70 (2.8)	36 (1.2)	106 (1.9)	0.000*
Cow milk	238 (9.4)	141 (4.8)	379 (6.9)	0.000*
Beef- mutton	96 (3.8)	62 (2.1)	158 (2.9)	0.000*
Cashew-peanut- soybean	58 (2.3)	23 (0.8)	81(1.5)	0.000*
Mango	37 (1.5)	13 (0.4)	50 (0.9)	0.000*
Wheat	103 (4.1)	65 (2.2)	168 (3.1)	0.000*
Number of Patients	**1 708**	**2 013**	**3 721**	
Trees	71 (4.2)	58 (2.9)	129 (3.5)	0.034*
Egg white/egg yolk	41 (2.4)	20 (1.0)	61 (1.6)	0.000*
Fish-prawn-crab	32 (1.9)	24 (1.2)	56 (1.5)	0.089

Note. Two-sided *P*-values: Chi-square test for gender specific positive rates to allergens. $^{*}P<0.05$.

mix, similar with that reported in HongKong, Kota Kinabalu, and Singapore[12-13]. It suggested that climate determines the types of flora and fauna in a particular geographical area and airborne and food allergens are affected[14]. In Harbin, the annual average temperature is low, and the average temperature in January ranges from 8.2 °F (-13.2 °C) to -12.6 °F (-24.8 °C). Therefore, aeroallergens such as house dust mite, short ragweed estragon and trees had no suitable environment to live and people have rare chances to contact these aeroallergens, and the positive rates to these aeroallergens are low. On the other hand, more indoor activities increases the contacts with cat epithelium/dog epithelium allergens, therefore the positive rate to this allergen is high.

The data showed that the positive rate to cow milk (6.9%) was relatively higher in Harbin, which was inconsistent with previous finding that the common food allergens in China were fish, shrimp, and crab[15-17].

One explanation might be that Harbin is an inland city and sea foods are not commonly consumed. On the other hands, the people in northern China often take more meat and grain as main energy source against cold climate, therefore, the positive rates to beef-mutton and wheat were relatively higher. Because mango is a rare fruit in Harbin, the related positive rate was lowest.

Milk protein allergy has become a worldwide public health problem. The prevalence of cow milk allergy is estimated to be between 2% and 3% in infants in the Netherlands[18]. In our study children had a higher positive rate to cow milk. This finding is different from the reports in most developed countries as well as in the southern region in China where the common food allergens are eggs for the children[19-20].

Whey protein and casein are the main allergens in milk allergy. The β-Lacto globulin, the most abundant content of whey protein in cow milk, is also the main allergen. There is no β-Lacto globulin in human milk, so human milk is naturally low allergic. Wang et al. reported that breast feeding rate dropped by 21.9% and mixed feeding rate and infant formula feeding rate increased by 20.9% and 0.9% in eight provinces (municipalities) in China from 2002 to 2009[21]. Nevertheless, it was reported that cow milk protein could be detected in breast milk[22-23], which indicated the decline of breast feeding rate might be related with the high sensitive rate to milk allergens, though further studies is needed.

Generally speaking, cow milk allergy occurs in early childhood and may affect child's diet. Due to the complexity of immune system, the diagnosis of food allergy of infant is difficult, therefore the sIgE detection is so important in the clinical diagnosis. The cause for high positive rate to milk in children in Harbin still needs further studies.

In analysis of age strata, some of allergens were related with age, some were not. We believe that first, an allergy disease is a reaction to a specific allergen, in which genetic predisposition may play a role[4]. In our study, 52 children aged<1 year were positive to 11 kinds of allergens except grass, mango, and fish-prawn-crab. During this early stage of growth, food type and physical activity are limited, so it can be speculated that allergy in these children was related with congenital allergic constitution.

Second, the positive rate to inhaled allergens was high in children aged>7 years, which might be explained by the increased exposure opportunities. In China young children usually go to elementary school at the age of 7 years and have junior high school and senior high school education when they are 13 and 17 years old, so the exposures to allergens increase, resulting in the higher positive rate to inhaled allergens among them. Adults have more contact with aeroallergens. In females over 50 years old the positive rates to house dust mite mix and mould mix increased and the rate to ragweed estragon decreased, which might also be due to the contacts with different allergens. Furthermore, immunity system develops with age and the sensitivity to allergens may be different.

The positive rates to cow milk and beef-mutton declined with age. In our study the positive rate to cow milk was 20.5% in age group 0-3 years and 17.2% in age group 4-6 years. This was mainly due to the sensitivity of children to specific food allergens which results in early allergic reaction in childhood and early detection of food allergens. For many patients, sIgE antibodies to foods appear within the 2 years old[24]. Early detection would help most patients avoid contact with allergens in daily life, which lead to desensitization to the food. At the same time, food tolerance appear along with the development of immune system, therefore the positive rate would decline. Most allergic diseases occurring in childhood eventually disappear in adulthood. Because the positive rates to fish-prawn-crab, cashew-peanut-soybean and mango were Less than 2.5%, the statistics differences could not been concluded.

This study indicated that it is necessary to strengthen the prevention of food allergy. Children aged≥7 years and adults should pay more attention to the prevention of inhaled allergic disease. Adults aged≥50 years should pay more attention to ventilation and house cleaning to prevent the contact with inhaled allergens.

The gender specific positive rates to allergens were different and this might be due to different chances of contacting different allergens[25-26] and the difference in immune function between males and females[27].

We found in this study that the positive rates of sIgE to different allergens had no significant season specific differences, which was inconsistent with those reported by other studied in China. Allergic disease is related with human immune system that is relatively stable during the adulthood. For example, if someone was sensitive to trees, the sIgE to trees in his serum could been detected by the AllergyScreen test though the amount was small, and trees would be allergens to him during his/her life time. No sIgE to trees would be detected temporarily after a severe allergic reaction and permanently after desensitization therapy. The seasonality of allergic diseases means that in a particular season the chances of contacting allergens increase, therefore the incidences of the diseases increase too. In common words, the pathogenesis of allergic disease is related with season, but whether a person is sensitive to a certain allergen is actually not related with season. For another example, a boy was diagnosed with allergic rhinitis and house dust mites mix was found to be the allergen, but at the same time his fish-shrimp-crab allergen test was also positive while the boy didn't eat fish, shrimp or crab recently. Therefore, there were no obvious symptoms of digestive tract or skin, but actually the boy was allergic to the allergen.

Allergic hypersensitivity may be classified as IgE-mediated or not[28]. IgE normally represents only a min amount in serum (about 5×10^{-5}mg/mL). When the allergens enter the body by either inhalation or ingestion, the specific B cells are induced and generate a response to secrete serum specific Immunoglobulin E (sIgE). A certain amount of sIgE result in the increase of serum total IgE, however, the increased total IgE level might be in the normal range due to the min amount. In our present study, serum total IgE level elevated in 63.3% of the patients. One explanation may be that when a person is not in a serious allergic reaction the amount of serum sIgE is low, which could not affect the total IgE levels obviously. Another reason was related with non IgE-mediated disease. Therefore the clinical diagnoses of allergic diseases would depend not only on the IgE detection but also on the clinical manifestations and the allergen test results.

In this study we found that the number of patients detecting sIgE has increased year by year and outpatients with extrinsic asthma, allergic rhinitis, eczema, atopic dermatitis, and food allergy increased with year (data not shown), which suggested that the prevalence of allergic disease in Harbin was high and further study is needed.

The actual prevalence of allergic disease is difficult to calculate because there are hundreds of allergens in the world, for instance, more than 170 foods have been reported to cause IgE-mediated reactions in the United States[24]. Only 14 common allergens were tested in our study due to the limited funds, therefore a negative result does not mean that the patients were not allergic to other allergens.

Some patients made self diagnosis according to allergen contact history and apparent clinical manifestations and some patients did not go to see doctors due to inconvenient transportation and medical cost. Therefore the number of patients who go to see doctor for allergens detection are limited. A number of IgE-detection test and allergy screen test reagents have been commercially available but their analytical and diagnostic performance needs to be evaluated systematically. Immunoblot method is a good assay for detection serum allergen sIgE with higher sensitivity and specificity. In our study the reagents of AllergyScreen test were imported from Germany and antigens of different allergens were packaged on the nitrate membrane. Therefore, whether the antigens are suitable for northern China people or not needs further assessment.

Because clinical symptoms of anaphylactic diseases are complex, some symptoms are obvious and some are not, allergen test is very important to avoid misdiagnosis. The specific IgE level may be different before and after allergic reactions and the results of AllergyScreen test also have differences in different stages of the disease. Generally speaking, when a patient is allergic to a special allergen, the sIgE increase and the level is related with the severity of the disease; but the degree of sIgE is not parallel with clinical symptoms, especially in serious allergic reaction, In serious allergic reaction, the sIgE is all consumed and false negative result may be obtained. Serum specific IgE test is a very important auxiliary examination to identify allergen, so the patient can get appropriate therapy and allergic disease can be prevented[29]. Although diagnosis of allergic disease mainly depend on allergen test, the comprehensive analysis on the patients' clinical symptoms and disease histories should be conducted too.

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